

Blood plasma chemistry in White Sea bearded seals across different age groups

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Abstract

Blood chemistry values are reported for the bearded seal species (*Erignathus barbatus barbatus* Erxleben, 1777) from the White Sea. 27 blood plasma indices are used to describe the state of the metabolism of proteins, carbohydrates, lipids, minerals (total protein, albumin, α -, β -, γ -globulins, urea, creatinine, uric acid, glucose, lactic acid, total lipids, triglycerides, cholesterol, calcium, phosphorus, sodium, potassium, magnesium, iron, copper, chlorides, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transferase, creatine kinase, alkaline phosphatase, lactate dehydrogenase) in 3-, 5- and 10-year-old seals. The values of the studied parameters are similar to published data for bearded seal populations in other Arctic regions, as well as being in close agreement with indicators of other pinniped species of the same age. The plasma content of total protein, albumin, α -, β -, γ -globulins, urea, uric acid, total lipids, calcium, magnesium, sodium, and chlorides is invariable in animals of the studied ages. In common with other pinnipeds and land-based mammals, age-related changes in metabolic rates in the studied animal groups are most pronounced in the activity level of key metabolism enzymes. While aspartate aminotransferase, alanine aminotransferase and γ -glutamyl transferase activity is higher in adult seals than in immature animals, alkaline phosphatase, creatine kinase and lactate dehydrogenase activity is lower. Changes in biochemical parameters of bearded seal blood at the ages of 3, 5 and 10 years indicate a catabolic orientation in metabolism at all studied development stages. Although more research is needed to investigate the biological significance of a number of blood indices, the data presented in this study provide initial baseline blood chemistry parameters for use in assessing the condition of individual seals, as well as informing monitoring and management efforts for wild seal populations.

Keywords

age differences, biochemical blood composition, bearded seals, pinniped metabolism

Introduction

Despite their broad distribution, bearded seals are among the least studied Arctic marine mammals. The bearded seal of the Atlantic subspecies (*Erignathus barbatus barbatus* Erxleben, 1777) inhabits the shallow coastal areas of the seas of the Arctic Ocean. The taxonomic isolation of the genus *Erignathus*, which occupies a special position in the series of ten-incisor seals, is confirmed by the serological and cytogenetic data: there are 34 chromosomes in the bearded seal karyotype, while all other true seals have 32 chromosomes, possibly indicating the earlier evolutionary occurrence of *Erignathus*. Along with other peculiarities of geographical distribution and biology, this fact allows us to suggest other special features at different levels of organisation in bearded seals (Anbinder 1980). In terms of research into this arctic pinniped species, individual works are mainly devoted to the study of distribution, abundance, reproduction biology, as well as some aspects of respiration physiology (Svetocheva et al. 2017; Svetochev and Kavtsevich 2019; Andersen et al. 1999; Charrier et al. 2013; Charmain et al. 2018). There is still practically no data in the literature on the metabolic characteristics of these animals as revealed by haematological studies: even in the relevant section of the CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation (Bossart et al. 2001), the bearded seal is not mentioned. Only recently has data on the blood composition of bearded seals from Alaska been published in connection with marine animal health assessment (Goertz et al. 2019). In a guidebook to Russian marine mammals (Burdin et al. 2009), it is noted that no studies specifically devoted to bearded seals have been carried out in Russia. To date, the Murmansk Marine Biological Institute of the Russian Academy of Sciences is the only institute to have obtained initial data on the composition and properties of blood cells of the population of this species inhabiting White Sea (Minzyuk et al. 2015). Although forming an integral part of this continuing line of research, the present work, which is devoted to a biochemical study of metabolic indicators in the blood plasma of bearded seals, represents a novel approach to studies of the White Sea bearded seal. At the

same time, the eco-biochemical monitoring reported in the study may be of interest for the assessment of changes in metabolism that normally take place prior to morphological and population deviations from the norm, thus permitting its application in the early diagnosis of changes taking place in the marine environment. Due to the impact of environmental factors representing a function of the developmental stage of organisms, the ontogenetic principle becomes an integral part of the system of eco-biochemical monitoring. Thus, in connection with the foregoing, the aim of this study was to establish reference values for bearded seals of different age groups.

Materials and methods

Blood of bearded seals was obtained during expeditions to the Onega Bay of the White Sea in July 2014–2015 from animals of the following age groups: 3 years (juvenile), 5 years (sexually mature), 10 years (adult). Animals were treated according to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986). Seals were captured around the marine coastline with a hoop net under manual restraint without previous immobilisation. All animals were apparently healthy (i.e. with no external indications of disease). Age determination for seals was assessed using body size and the condition of claws on the front flippers. Blood samples were taken from the extradural intravertebral vein as described in (Geraci and Smith 1975) with a spinal needle (1.2 × 90 mm), then delivered immediately into blood sample tubes using heparin as an anticoagulant. Plasma was separated by centrifuging for ten minutes at a rate of 1500 rpm. The content of the main indicators of metabolism of proteins, lipids, carbohydrates and mineral substances in blood plasma was determined using conventional laboratory methods (Danilova 2003). The obtained data were processed using statistical methods (Zaitsev 1991). To assess the differences between samples, Student's t-test with a significance level of $p \leq 0.05$ was used. The study results are presented in the form of average values and standard errors ($M \pm m$).

Results

Bearded seal blood plasma samples were analysed for 27 metabolic indicators including proteins, lipids, carbohydrates and mineral substances. The study results are presented in Table 1. The data show that a number of indicators have similar values in animals of different age groups. Such indicators include total protein, albumin, globulins, urea, uric acid, total lipids, calcium, magnesium, sodium, chlorides. However, other indicators exhibit multidirectional age-related changes (Fig. 1).

The biochemical composition of the blood of 5-year-old bearded seals differs significantly from that of 3-year-old animals in 8 out of 27 studied parameters. Levels of glucose, triglycerides, potassium, and γ -glutamyl transferase activity are higher, while creatinine and copper levels, along with aspartate ami-

notransferase and creatine kinase activity, are lower in 5-year-old seals as compared to 3-year-old animals.

While adult seals (10-year-olds) differ in 11 biochemical blood parameters in comparison with both 3-year-old and 5-year-old animals, the lists of indicators differ (Fig. 1). Levels of glucose, lactic acid, cholesterol, as well as γ -glutamyl transferase and alanine aminotransferase activity, are higher in adult seals as compared to 3-year-old animals, while triglycerides, copper, phosphorus, as well as creatine kinase, alkaline phosphatase and lactate dehydrogenase activity, are lower. Levels of creatinine, lactic acid, cholesterol, copper, along with aspartate- and alanine aminotransferase activity, are higher, while triglycerides, phosphorus and iron, along with alkaline phosphatase and lactate dehydrogenase activity, are present in lower quantities in adult seals as compared to 5-year-old animals.

Table 1. Biochemical indicators of blood plasma in bearded seals of different ages

N	Indicators	Animal age, years		
		3 (n = 4)	5 (n = 4)	10 (n = 2)
1	Total protein, g/l	71.01 ± 6.15	75.64 ± 5.26	67.70 ± 4.70
2	Albumin, g/l	31.10 ± 4.12	34.11 ± 2.00	31.01 ± 3.50
3	α -globulins, g/l	6.07 ± 1.44	6.44 ± 1.14	6.20 ± 0.86
4	β -globulins, g/l	14.70 ± 1.12	17.16 ± 2.72	13.65 ± 0.95
5	γ -globulins, g/l	19.13 ± 4.60	17.41 ± 2.68	16.84 ± 2.15
6	Urea, mmol/l	8.07 ± 0.73	8.51 ± 0.18	8.69 ± 0.27
7	Creatinine, μ mol/l	148.64 ± 6.11	102.86 ± 4.18 ^a	154.31 ± 5.96 ^b
8	Uric acid, μ mol/l	92.25 ± 15.83	84.24 ± 9.55	109.32 ± 12.45
9	Glucose, mmol/l	1.91 ± 0.11	5.17 ± 0.07 ^a	5.20 ± 0.25 ^a
10	Lactic acid, mmol/l	14.65 ± 0.95	15.75 ± 1.42	25.48 ± 3.06 ^{a,b}
11	Total lipids, g/l	3.83 ± 0.79	4.11 ± 0.76	4.44 ± 0.41
12	Triglycerides, mmol/l	0.32 ± 0.01	1.14 ± 0.16 ^a	0.13 ± 0.02 ^{a,b}
13	Cholesterol, mmol/l	1.87 ± 0.34	1.60 ± 0.25	3.84 ± 0.40 ^b
14	Calcium, mmol/l	2.99 ± 0.35	2.80 ± 0.27	2.75 ± 0.36
15	Phosphorus, mmol/l	3.55 ± 0.43	3.61 ± 0.40	2.15 ± 0.10 ^{a,b}
16	Magnesium, mmol/l	0.70 ± 0.09	1.06 ± 0.18	0.89 ± 0.13
17	Iron, μ mol/l	60.25 ± 11.17	71.71 ± 6.12	43.50 ± 5.24 ^b
18	Copper, μ mol/l	23.19 ± 4.20	5.22 ± 0.17 ^a	10.43 ± 1.06 ^{a,b}
19	Chlorides, mmol/l	117.97 ± 19.71	101.58 ± 7.43	105.96 ± 3.13
20	Sodium, mmol/l	155.76 ± 3.87	159.40 ± 4.77	160.10 ± 4.40
21	Potassium, mmol/l	4.14 ± 0.24	4.87 ± 0.15 ^a	5.04 ± 0.37
22	Aspartate aminotransferase, IU/l	238.04 ± 24.63	116.98 ± 9.68 ^a	251.42 ± 18.10 ^b
23	Alanine aminotransferase, IU/l	36.67 ± 1.42	39.29 ± 6.10	65.48 ± 5.46 ^{a,b}
24	γ -glutamyl transferase, IU/l	12.74 ± 0.42	17.37 ± 0.75 ^a	15.63 ± 0.58 ^a
25	Alkaline phosphatase, IU/l	249.97 ± 20.11	261.92 ± 19.40	158.53 ± 9.65 ^{a,b}
26	Creatine kinase, IU/l	400.32 ± 28.70	309.53 ± 21.45 ^a	260.50 ± 22.17 ^a
27	Lactate dehydrogenase, IU/l	2333.20 ± 120.36	2287.04 ± 210.50	1409.48 ± 112.20 ^{a,b}

Note. n – number of animals; symbols a and b indicate statistically significant differences when comparing the indicators of older animals with those at the age of 3 years and 5 years, respectively.

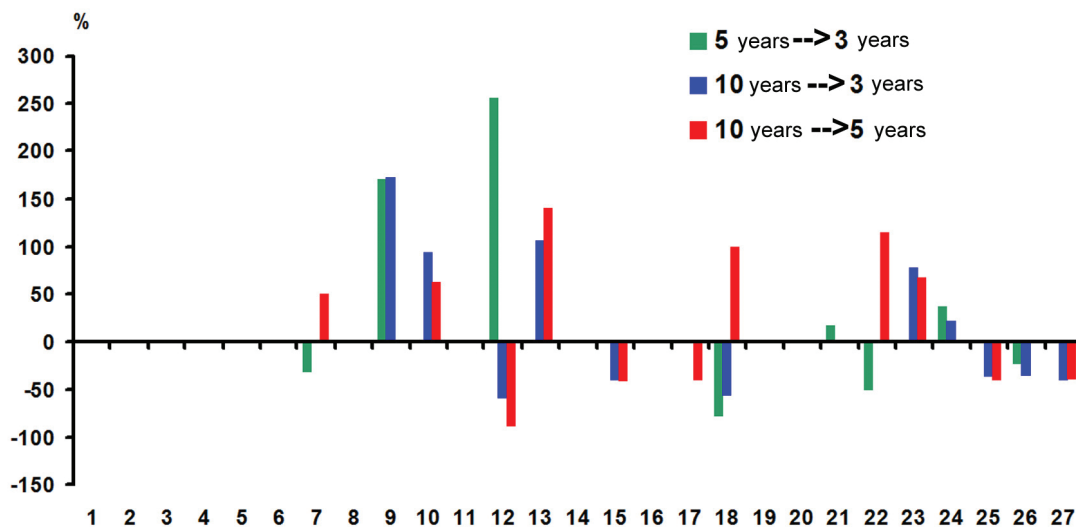


Fig 1. Changes in biochemical parameters in the blood plasma of bearded seals of various ages. The numbers on the category axis correspond to the numbers of indicators in Table 1. Statistically significant differences are expressed as a percentage in relation to the indicators of the comparison group, taken as 100%

Discussion

On the whole, the biochemical indices of the metabolism of bearded seals correspond to the values characteristic of other pinniped species the same age (Bossart et al. 2001; Boily et al. 2006). Age-related changes in individual indicators demonstrate a similar pattern, proceeding according to the same principles as observed in terrestrial mammals. As can be seen in Fig. 1, age-related changes in glucose, triglycerides, cholesterol vary significantly. It is known (Bossart et al. 2001) that changes in diet can affect these blood parameter levels in both marine and terrestrial mammals.

Age-related changes in enzyme activity deserve special attention due to these compounds integrating all metabolic links. Differences were found in the activity of all studied enzymes – aminotransferases, γ -glutamyl transferase, alkaline phosphatase, creatine kinase and lactate dehydrogenase.

Aminotransferases – aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) – support transamination in the organism. This process plays a key role in intermediate exchange, ensuring the synthesis and decomposition of individual amino ac-

ids. At the same time, AlAT more extensively reflects the level of anabolism, while AsAT indicates the intensity of catabolism. The aggregate of conjugated activities of AlAT \leftrightarrow AsAT represents a common marker of all metabolism in simplified form (Rosliy and Vodolazhskaya 2010). A characteristic feature of the state of transaminases within the studied age groups is the predominance of AsAT activity over AlAT, indicating a catabolic orientation of metabolism.

γ -Glutamyltransferase (GTP) is an amino acid membrane transport enzyme associated with the cell membranes of many organs (liver, heart, muscles, kidneys, pancreas). The level of this enzyme in the blood of bearded seals is comparable with that of other pinniped species, along with terrestrial mammals and humans. In adult specimens, GTP activity is higher than in young seals (Table 1), indicating the activation of gluconeogenesis, i.e. obtaining the main energy substrate (glucose) from non-carbohydrate sources, mainly amino acids. GTP acts as an active transporter of amino acids to tissues from various protein pools (blood, cells, connective tissue, muscles and other organs). The above-noted catabolic orientation in the state of

transaminases confirms the activation of gluconeogenesis in older seals.

Alkaline phosphatase (ALP) found in the blood plasma of adult animals is of hepatic origin; in the early stages of ontogenesis, the bone fraction is present in a significant amount, with this non-specific enzyme cleaving the phosphate residue from any compounds in which it is present. In marine mammals, the activity of alkaline phosphatase is higher in all age groups as compared with terrestrial mammals (Bossart et al. 2001; Boily et al. 2006). There is evidence that the level of alkaline phosphatase in the plasma of marine mammals is directly correlated with the intensity of anabolic processes in the body, on the basis of which the concentration of the enzyme can be used as an indicator of the nutritional status of animals, as well as the differentiation of catabolic and anabolic conditions (Dover et al. 1993). The latter is also confirmed in the present study: in the 10-year-old group, the activity of alkaline phosphatase in bearded seals has decreased significantly as compared to younger animals (Table 1). At the same time, the concentration of inorganic phosphorus in the blood plasma is observed to decrease.

Creatine kinase (CK) catalyses the conversion of creatine to creatine phosphate. The metabolic role of this enzyme is to serve as a macroergic phosphate carrier across the mitochondrial membrane. In addition, CK acts as a membrane protector, regulating the structure of the cell membrane following damage (Rosliy and Vodolazhskaya 2010). Normally, the level of CK in mammals can vary widely – from units to several thousand IU/l (Aktas et al. 1993; Boyd 1984; Bossart et al. 2001). The activity of CK decreases with age, as shown in our study (Table 1) as well as from literature sources (Aktas et al. 1993). Scarce information on CK activity in marine mammals is mainly related to cetaceans, as well as walruses, common seals and manatees (Bossart et al. 2001). CK activity in bearded seals is shown to be in the range of 260–400 IU/l, which is comparable to that of manatees, but 3–5 times higher than that of walruses. Conversely, in adult common seals, CK activity can reach 2351 IU/l (Bossart et al. 2001). Evidence from studies of terrestrial animals (Boyd 1984; Aktas et al. 1993) and humans (Danilova 2003; Rosliy and Vodolazhskaya

2010) shows that the influence of CK activity is also determined by the level of motor activity.

The enzyme lactate dehydrogenase (LDH) has consistently high activity – up to 250 IU/l in humans (Rosliy and Vodolazhskaya 2010), 938 IU/l in terrestrial mammals (Boyd 1984), 584 IU/l in cetaceans and 1684 IU/l in pinnipeds (Bossart et al. 2001). In our study, LDH activity is also shown to be high in bearded seals: in 3–5-year-old animals, it is more than 2000 IU/l, decreasing to 1409.48 ± 112.20 IU/l in 10-year-old specimens. At the same time, an increase ($p < 0.02$) in the plasma of the concentration of lactic acid, a reaction product catalysed by LDH, is noted.

Despite the stable level of total lipids in the blood plasma of seals of different ages, some lipid metabolism indicators vary across the different studied bearded seal age groups. Thus, for example, the triglyceride content of the blood plasma of 5-year-old seals significantly exceeds that of younger animals (Figure 1). However, in adult seals, the triglyceride content decreases again, falling to even lower levels than those observed in 3-year-old seals. The measured cholesterol content of blood plasma is twice as high in adult bearded seals as in younger animals (Figure 1). Although triglyceride and cholesterol values can vary widely with dietary changes (Bossart et al. 2001), the greatest influence on the level of these indicators in the period of maturity is probably the metabolism of steroid hormones.

Age-related changes in mineral metabolism indices in bearded seals are similar to those found in other mammals, both marine (Bossart et al. 2001; Goertz et al. 2019) and terrestrial (Boyd 1984; Maksimov et al., 2014). In our study, the levels of calcium, magnesium and sodium in the blood plasma of seals are similar across the different age groups (Table 1). The content of phosphorus, iron, copper, while high in the blood plasma of young seals, decreases in adult animals (10 years old). Evidently, this is associated with a decrease in metabolic rate due to the completion of the structural and functional formation of the organism and regulatory mechanisms. Significant age-related changes in the plasma copper content of bearded seals should be noted: the blood plasma copper content of 3-year-old seals is more than four times higher than

that of 5-year-olds and more than twice that of adults. This reflects the need for a high copper content in young animals to ensure haematopoiesis and oxidative processes in the connective tissues, as well as osteogenesis, in which copper performs a regulatory function (Zaychik and Churilov 2002; Samokhin 2003).

Conclusion

This study presents data on blood plasma chemistry in wild bearded seals from the White Sea for the first time. Changes in biochemical parameters of bearded seal blood at the ages of 3, 5 and 10 years indicate

a catabolic orientation of animal metabolism in all studied age groups. Age-related changes in metabolic rates in the studied groups are most pronounced in the activity of key metabolism enzymes, as is the case with other pinnipeds and terrestrial mammals. Although more research is needed to investigate the biological significance of a number of blood parameters, establishing reference values is an important step in the health assessment of wild seal populations.

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